Cell Cryopreservation – Quantitative Cell Biology

In vitro experiments using cells derived from marine mammals will play a critical role in understanding marine mammalian physiology as well as the effects of environmental conditions. NIST performed a number of measurements using automated fluorescence microscopy on a terrestrial mammalian cell line to determine baseline values for variety of parameters, from cellular morphology to necrosis. It was shown that it is possible to obtain a consistent, day-to-day baseline under highly controlled culture conditions.

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Today's cryopreservation protocols, developed in the 1950s and 1960s, do not support the functional requirements necessary for modern cell-preservation needs in pharmaceutical, drug discovery, basic science, and toxicological studies. This year, a number of measurements were performed using automated fluorescence microscopy on a terrestrial mammalian cell line (NIH 3T3) determining baseline values for parameters such as cellular morphology as well as rates of apoptosis, necrosis, adherence, metabolism, and proliferation under highly reproducible culture conditions. It was shown that it is possible to obtain consistent, day-to-day baseline results for all of the parameters tested under highly controlled culture conditions.

Marine mammals are an important part of both the economy and ecology of coastal environments, and a greater understanding of their physiology could have enormous implications for the improvement of human health. Despite their importance, experimentation with living marine mammals is difficult for both practical and political reasons. Thus, it is clear that *in vitro* experiments using cells derived from marine mammals will play a critical role in understanding marine mammalian physiology as well as the effects of environmental conditions. In addition, development of specific marine cell lines will pave the way for enhancing basic insights into fundamental cellular processes in marine model organisms as well as allowing marine biopharmaceutical production.

Using automated fluorescence microscopy on a carefully cultured mammalian cell line, NIST researchers demonstrated that it is possible to obtain reproducible baseline results for a variety of parameters important to cell preservation and storage.

These baseline values will next be compared to assay results obtained post-thaw in order to ensure that the freeze/thaw process has not resulted in the inadvertent selection of a subset of cells that are dissimilar to the original population. Knowledge of the expected results of these assays will help identify tools that aid in determining the optimal storage, preservation, and propagation conditions that ensure retention of selected phenotypic properties in a marine mammal cell line.

APOPercentage is a dye that is internalized only by cells containing phosphatidylserine in the outer leaflet of the plasma membrane – an early indicator of apoptosis. Comparison of the number of nuclei with the number of apoptotic cells (arrow) gives the rate of apoptosis among the population.